

**WEST**

Generate Collection

Print

L1: Entry 10 of 89

File: USPT

Aug 27, 2002

DOCUMENT-IDENTIFIER: US 6440418 B1

TITLE: Methods of treating autoimmune diseases with gp39-specific antibodies

**Brief Summary Text (12):**

Also, a ligand for CD40, gp39 (also called CD40 ligand or CD40L) has recently been molecularly cloned and characterized (Armitage et al, Nature, 357:80-82 (1992); Lederman et al, J. Exp. Med., 175:1091-1101 (1992); Hollenbaugh et al, EMBO J., 11:4313-4319 (1992)). The gp39 protein is expressed on activated, but not resting, CD4<sup>sup.</sup> Th cells. Spriggs et al, J. Exp. Med., 176:1543-1550 (1992); Lane et al, Eur. J. Immunol., 22:2573-2578 (1992); and Roy et al, J. Immunol., 151:1-14 (1993). Cells transfected with gp39 gene and expressing the gp39 protein on their surface can trigger B cell proliferation and, together with other stimulatory signals, can induce antibody production. Armitage et al, Nature, 357:80-82 (1992); and Hollenbaugh et al, EMBO J., 11:4313-4319 (1992). In particular, the ligand for CD40, gp39, has been identified for the mouse (Noelle et al, Proc. Natl. Acad. Sci. USA, 89:6550 (1992); Armitage et al, Nature, 357:80 (1992)) and for humans (Hollenbaugh et al, EMBO J. 11:4313 (1992); Spriggs et al, J. Exp. Med., 176:1543 (1992)). gp39 is a type II membrane protein and is part of a new gene super family which includes TNF- $\alpha$ , TNF- $\beta$ , and the ligands for FAS, CD27, CD30 and 4-1BB.

**Drawing Description Text (5):**

FIG. 3 contains FACS of non-transfected CHO cells and a gp39 transfectant. FACS analysis of non-transfected CHO cells and a gp39 transfectant. 1.times.10<sup>sup.6</sup> cells were treated with the mouse anti-gp39 antibody 24-31 and then with a goat-anti-mouse IgG-FITC conjugate (Southern Biotechnology Associates). The samples were analyzed on FACScan (Becton Dickinson).

**Detailed Description Text (42):**

In particular, the inventors elected to express the subject humanized V.sub.L and V.sub.H sequences, as well as the native (unmodified) V.sub.L and V.sub.H sequences derived from 24-31 in CHO cells using the N5KG1 expression vector which contains human Kappa and human gamma 1 constant regions. The N5KG1 expression vector is depicted schematically in FIG. 1. As hoped, the chimeric antibody derived from 24-31, when expressed in CHO cells binds gp39 (by demonstrated binding to CHO-gp39 transfectant). Also, several humanized antibodies of the invention derived from 24-31 when expressed using this vector system resulted in functional (gp39 binding) antibodies.

**Detailed Description Text (59):**

Generation of a gp39 CHO Transfectant Cell Line

**Detailed Description Text (60):**

Recently, a CHO transfectant that constitutively expresses cell-surface gp39 was generated to use as a reagent for the humanized anti-gp39 24-31 binding studies proposed in this application. The full-length gp39 gene (Hollenbaugh et al, Immunol. Rev., 138:23 (1994)) was amplified by polymerase chain reaction (PCR) of phytohemagglutinin-activated human PBL and cloned into IDEC's INPEP4 vector under the transcriptional control of the cytomegalovirus (CMV) promoter and enhancer elements. A CHO transfectant was established and amplified in 50 nM methotrexate. The transfectant, 50D4, was shown to express cell-surface gp39 by ELISA (data not shown) and FACS analysis (FIG. 3).

**Detailed Description Text (66):**

The anti-gp39 24-31 V.sub.k and V.sub.H gene segments were cloned and sequenced. Following analyses of their sequences, humanized versions of the V region gene segments were designed. The corresponding DNA sequences were synthesized and cloned into a high-level expression vector containing human constant region genes. A CHO transfectant producing the humanized 24-31 antibody is then established. To confirm that the humanized version of the anti-gp39 antibody retains its gp39 binding affinity, the relative affinities of the murine and humanized antibodies were compared in direct binding and competition assays. In addition, the ability of the humanized 24-31 to block CD40 binding to gp39 and to inhibit T cell-dependent antibody production is evaluated.

#### Detailed Description Text (73):

Humanized versions containing the most preferred humanized 24-31 V.sub.k and V.sub.H sequences identified in Tables 1 and 2 as humanized V.sub.L and V.sub.H (1) were synthesized. Specifically, four pairs of overlapping, complementary oligonucleotides (oligos) encoding the above-identified humanized V.sub.k or V.sub.H regions were synthesized (Midland Chemicals) and purified by denaturing polyacrylamide gel electrophoresis (Ausubel et al, Current Protocols in Molecular Biology, Vol. 2, Greene Publ. Assoc. (1992)). Each oligo is approximately 100 bases in length and overlap by 20 bases the adjacent complementary oligonucleotide. The V.sub.k and V.sub.H 5' oligos contain Bgl II and Sal I cloning sites and the 3' oligos possess Bsi WI and Nhe I cloning sites, respectively. Each variable region gene segment was assembled from the synthetic oligos, diagrammed below, using the following procedure (summarized in Watson et al, Recombinant DNA, 2nd Ed., Scientific. Amer. Books, NY, N.Y. (1992)). Complementary oligo pairs (A+E, B+F, C+G, D+F) were kinased using 300 pmoles of each primer and T4 polynucleotide kinase (Promega) according to the manufacturer's protocol. The oligos were annealed by heating to 95.degree. C. and slow cooling to room temperature. The annealed oligo pairs were ligated (A/E with B/F and C/G with D/H) utilizing 6 units T4 DNA ligase (New England Biolabs). After digestion with the appropriate 5' or 3' cloning site restriction endonuclease, the approximately 200 base pair DNA fragments were purified by electroelution following polyacrylamide gel electrophoresis (Sambrook et al, (Id.)). The synthetic gene fragments were then inserted into IDEC's proprietary high-level expression vector, N5KG1, under the transcriptional control of the CMV promoter and enhancer elements. The ligation reaction contains the 2 gel-purified fragments (A/B/F and C/G/D/H) and N5KG1 at a molar ratio of 100:100:1, respectively. After transformation of XL1-blue cells, plasmid DNA was prepared and the sequences of the synthetic gene segments confirmed. The resulting construct, h24-31, encodes the humanized 24-31 V region segments and human kappa and gamma 1 constant regions. As indicated, this antibody contains the humanized variable heavy and humanized variable light sequences identified in Table 1 and Table 2 as the "(1)" sequences, which are predicted to provide for humanized antibody having optimal gp39 properties. In addition, a construct was generated which contains V.sub.L #2 in combination with V.sub.H #1 (version 2 of humanized 24-31). Similar constructs utilizing IDEC's proprietary vectors have been used for high-level expression of IDEC's anti-CD20 (Reff et al, Blood, 83:425 (1994)) and anti-CD4 (Newman et al, Biol. Technology, 10:1455 (1992)) antibodies. ##STR1##

#### Detailed Description Text (80):

The humanized anti-gp39 24-31 antibody is evaluated initially for direct binding to cell surface gp39 expressed on 50D4, the gp39 CHO transfectant described in Example 5. Supernatants from the G418-resistant h24-31 CHO transfectants that produce immunoglobulin are tested for binding to 50D4 cells and, as negative control, to CHO cells. In this assay 50D4, 1.times.10.sup.5 /well, are bound to the bottom of 96 well, poly-L-lysine coated polystyrene plates. The cells are fixed in 0.5% glutaraldehyde in phosphate buffered saline (PBS) for 15 minutes. Plates coated with CHO cells are generated similarly. The cell culture supernatants are added and antibody binding measured using a HRP-conjugated goat anti-human IgG, as described above.

#### Detailed Description Text (84):

After establishing that humanized anti-gp39 binds to gp39, an assay is effected to confirm that the humanized anti-gp39 retains its ability to block the binding of the ligand to its receptor. For this purpose, activated human peripheral blood T cells, or the gp39-transfected CHO cells, 50D4, are pretreated with graded concentrations

of murine 24-31 or with humanized 24-31 for 15 minutes at 4.degree. C. Following this preincubation, CD40-Ig-biotin is added and the binding determined by flow cytometry using PE-avidin. Concentrations of mabs to achieve a 50% reduction in CD40-Ig binding are determined.

**WEST**☐

Generate Collection

Print

L1: Entry 17 of 89

File: USPT

May 21, 2002

DOCUMENT-IDENTIFIER: US 6391637 B1

TITLE: Use of CD40 ligand, a cytokine that binds CD40, to stimulate hybridoma cells

Abstract Text (1):

There is disclosed a polypeptide (CD40-L) and DNA sequences, vectors and transformed host cells useful in providing CD40-L polypeptides. More particularly, this invention provides isolated human and murine CD40-L polypeptides that bind to the extracellular binding region of a CD40 receptor. Also disclosed are methods of simulating hybridoma cells to increase monoclonal antibody production by administering a CD40 ligand polypeptide that stimulates B cell proliferation.

Detailed Description Text (160):

This example describes purification of trimeric murine CD40L from supernatant fluid from transfected CHO cells. A CHO cell line expressing muCD40LT was maintained in suspension in spinner-flask cultures. For production, the cells were centrifuged and resuspended into a controlled 3 liter bioreactor in serum-free medium. Oxygen, agitation and pH were controlled for at 40% dissolved O.sub.2 (relative to air saturation), 150 RPM and 7.2, respectively. The culture was harvested after nine days. A total volume of approximately 160 ml of supernatant fluid from the bioreactor was dialyzed overnight at 4.degree. C. against 4 L of 20 mM Tris pH 7.5 buffer containing 150 mM NaCl, and then adjusted to 1M (NH.sub.4).sub.2 SO.sub.4 by the addition of solid (NH.sub.4).sub.2 SO.sub.4. Dialysis accomplished the removal of low-molecular weight contaminants; other techniques will also be useful for this purpose, for example, constant volume diafiltration.

**WEST**

Generate Collection

Print

L1: Entry 22 of 89

File: USPT

Jan 22, 2002

DOCUMENT-IDENTIFIER: US 6340459 B1

TITLE: Therapeutic applications for the anti-T-BAM (CD40-L) monoclonal antibody 5C8 in the treatment of reperfusion injury in non-transplant recipients

**Brief Summary Text (4):**

Interestingly, recent studies indicate that CD40 expression has a broader cellular distribution than originally described. CD40 has been shown to be expressed on monocytes (19), dendritic cells (22), epithelium (23, 21), basophils (24), and Hodgkin's tumor cells (25). Moreover, various cytokines can regulate CD40 expression on non-B cells. CD40 expression on thymic epithelial cells is upregulated by IL-1.alpha., TNF-.alpha. or INF-.gamma. (21). INF-.gamma., in addition to IL-3 or GM-CSF, similarly upregulates CD40 expression on monocytes (19). Ligation of CD40 in the presence of INF-.gamma. and IL-1.alpha. stimulates GM-CSF production by thymic epithelial cells (21). In addition, CD40L expressing transfectants induce tumoricidal activity by monocytes and, in the presence of INF-.gamma., GM-CSF or IL-3, stimulate monocytes to secrete TNF-.gamma., IL-6 or IL-8 (19).

**Brief Summary Text (7):**

It can now be reported that normal human endothelial cells also express CD40 in situ and CD40L-CD40 interactions induce endothelial cell activation in vitro. Frozen sections from normal spleen, thyroid, skin, muscle, kidney, lung or umbilical cord were studied for CD40 expression by immunohistochemistry. Endothelial cells from all tissues studied express CD40 in situ. Moreover, human umbilical vein endothelial cells (HUVEC) express CD40 in vitro and rIFN-.gamma. induces HUVEC CD40 upregulation. CD40 expression on HUVEC is functionally significant because CD40L.sup.+ Jurkat T cells upregulate HUVEC CD54 (ICAM-1), CD62E (E-selectin) and CD106 (VCAM-1) expression in vitro in a manner inhibited by anti-CD40L mAb 5C8. Additionally, CD40L expressing 293 kidney cell transfectants, but not control transfectants, also upregulate CD54, CD62E and CD106 expression on HUVEC. These results demonstrate that CD40L-CD40 interactions induce endothelial cell activation in vitro. It is shown for the first time that CD40L expressed on the surface of T cells induces activation of CD40L+ endothelial cells and that this activation is inhibited by an anti-CD40L monoclonal antibody. Moreover, these results demonstrate a mechanism by which activated CD40L+ T cells augment inflammatory responses in vivo by upregulating the expression of endothelial cell surface adhesion molecules.

**Drawing Description Text (6):**

FIG. 5. Transfection of CD40L confers the capacity to upregulate SM fibroblast CD54 (ICAM-1) and CD106 (VCAM-1) expression. Shown are bar graphs indicating CD54 or CD106 MFI on SM fibroblasts following culture for 24 hours with media, CD40L.sup.+ D1.1 cells, CD40L.sup.- B2.7 cells or CD40L.sup.+ B2.7 transfectants, as indicated. CD54 and CD106 expression were determined by two-color FACS analysis as in FIG. 4. The background MFI of an isotype control mAb is subtracted from each value. The experiment shown is representative of 2 similar experiments performed.

**Drawing Description Text (7):**

FIG. 6A. Effect of CD40L-CD40 interactions on fibroblast IL-6 secretion. Shown are bar graphs indicating sup.3 H-thymidine incorporation by the IL-6 indicator cell line B9 following the additions of supernatants (final dilution 1:60) from SM fibroblasts cultured with media alone, CD40L.sup.+ D1.1 cells in the presence or absence of anti-CD40L mAb 5C8 or control mAb Pl.17, CD40L.sup.- B2.7 cells or CD40L.sup.+ B2.7 transfectants. The proliferative responses of B9 cells cultured with control supernatants from D1.1 cells, B2.7 cells or CD40L.sup.+ B2.7

transfectants were 1136 cpm (+-.113), 2398 cpm (+-.263) and 1131 cpm (+-.56). Similar results were obtained with 3 additional SM fibroblast lines.

Drawing Description Text (9):

FIG. 7. Effect of CD40 ligation on SM fibroblast proliferation. Shown are bar graphs from 2 separate experiments demonstrating SM fibroblast .sup.3 H-thymidine incorporation following coculture in 1% FM with mitomycin-C treated CD40L- Jurkat B2.7 cells or CD40L.sup.+ Jurkat B2.7 transfectants for 48 hours. Where indicated, CD40L.sup.+ Jurkat B2.7 transfectants were pretreated with anti-CD40L mAb 5C8 (5 .mu.g/ml) or P1.17 control mAb (5 .mu.g/ml) prior to the addition to fibroblasts. In the experiment studying RA.5 proliferation, the proliferation of CD40L.sup.- Jurkat B2.7 cells or CD40L.sup.+ Jurkat B2.7 transfectants was 51.+-.7 cpm and 39.+-.3 cpm, respectively. In the experiment studying OA.6 proliferation, the proliferation of CD40L.sup.- Jurkat B2.7 cells or CD40L.sup.+ Jurkat B2.7 transfectants was 243.+-.5 cpm and 453.+-.95 cpm, respectively. Background proliferation is subtracted in coculture experiments. Also shown are the proliferative responses of fibroblasts following culture in 1% FM or 10% FM. Similar results were obtained in 3 additional experiments. Error bars show observed error.

Drawing Description Text (10):

FIG. 8. Effect of rINF-.gamma. on CD40L mediated SM fibroblast proliferation. Shown are bar graphs demonstrating SM fibroblast .sup.3 H-thymidine incorporation following coculture in 1% FM with mitomycin-C treated CD40L.sup.- Jurkat B2.7 cells or CD40L.sup.+ Jurkat B2.7 transfectants for 48 hours. Where indicated, SM fibroblasts were pretreated for 18 hours with rINF-.gamma. (1000 U/ml) prior to the addition of mitomycin-C treated CD40L.sup.- B2.7 cells or CD40L.sup.+ B2.7 transfectants. SM fibroblast proliferation was determined as outlined in Materials and Methods for First Series of Experiments. Background proliferation of CD40L.sup.- Jurkat B2.7 cells and CD40L.sup.+ Jurkat B2.7 transfectants was 185.+-.66 cpm and 65.+-.5 cpm, respectively. Background proliferation is subtracted in coculture experiments. Also shown are the proliferative responses of fibroblasts following culture in 1% FM or 10% FM. Similar results were obtained in 2 additional experiments. Error bars show observed error.

Drawing Description Text (17):

FIGS. 15A-L. Effect of CD40L expressing 293 kidney cell transfectants on HUVEC CD54, CD62E and CD106 expression. Shown are two-color contour graphs demonstrating the effects on HUVEC CD54, CD62E and CD106 expression following culture with media, CD40L.sup.+ Jurkat D1.1 cells, CD8.sup.+ 293 kidney cell transfectants or CD40L.sup.- 293 kidney cell transfectants for 6 hours. The X-axis demonstrates UEA-1 expression and the Y-axis demonstrates CD54 (left panel), CD106 (middle panel) or CD62E (right panel) expression. The numbers in the upper right hand corner of each graph indicates the percentage of UEA-1.sup.+ cells expressing CD54, CD106 or CD62E, as indicated (background staining of control mAb is subtracted for each value). Shown is representative of 3 similar experiments with different HUVEC lines.

Detailed Description Text (67):

D1.1 is a Jurkat T cell subclone that constitutively expresses CD40L (3, 21). B2.7 is a CD40L.sup.- Jurkat subclone (3, 21). CD40L.sup.+ Jurkat B2.7 transfectants expressing full length CD40L protein were generated as previously reported (20).

Detailed Description Text (73):

To determine the effect of CD40 ligation on the expression of fibroblast cell surface molecules, fibroblasts were cultured in 6 well plates as described above. When the fibroblasts were near confluence 1.times.10.sup.6 CD40L.sup.+ Jurkat D1.1 cells, CD40L.sup.- Jurkat B2.7 cells or CD40L.sup.+ Jurkat B2.7 transfectants were added to the culture. Where indicated, D1.1 cells were pretreated with anti-CD40L mAb 5C8 (10 .mu.g/ml) or isotype control mAb P1.17 (10 .mu.g/ml) prior to the addition to fibroblasts. After 24 hours the cells were collected by trypsinization and two-color FACS analyses performed.

Detailed Description Text (74):

For studies determining the effect of CD40 ligation on fibroblast proliferation, approximately 5.times.10.sup.3 cells were added to flat bottom 96 well plates (Nunc) in 10% FM. After 18 hours the media was changed to 1% FM and rINF-.gamma. 1000 U/ml

added to the indicated cells. After an additional 18 hours, 1.times.10.sup.5 mitomycin-C (Sigma) treated CD40L.sup.+ Jurkat B2.7 transfectants or CD40L Jurkat B2.7 cells in 1% FM were added to the fibroblasts. Anti-CD40L mAb 5C8 (5 .mu.g/ml) or control mAb P1.17 (5 .mu.g/ml) were also added to some wells as indicated. 10% FM was added to some cells as a control for the induction of SM fibroblast proliferation. Cultures were maintained for an additional 48 hours and pulsed with 1 .mu.Ci .sup.3 H thymidine for the last 18 hours of the experiment. Following trypsinization, .sup.3 H thymidine incorporation was determined by harvesting onto glass fiber filter strips (Cambridge Technologies, Watertown, Mass.) and scintillation counting (BetaCounter, Pharmacia).

#### Detailed Description Text (75):

To determine the effect of CD40 ligation on IL-6 production, a bioassay utilizing the IL-6 responsive murine B cell line B9 was performed (22). Equal numbers of fibroblasts in 10% FM were seeded in 96 well plates as mentioned above. After adhering overnight, 1.times.10.sup.5 mitomycin-C treated CD40L.sup.+ Jurkat D1.1 cells, CD40L Jurkat B2.7 cells or CD40L.sup.+ Jurkat B2.7 transfectants were added to the fibroblasts. Where indicated, D1.1 cells were pretreated with anti-CD40L mAb 5C8 (10 .mu.g/ml) or control mAb P1.17 (10 .mu.g/ml). Control wells consisted of Jurkat cells cultured alone. After 48 hours, serial dilutions of fibroblast or control supernatants or rIL-6 were added to 7.5.times.10.sup.3 B9 cells in 96 well plates. B9 cells were maintained in culture for 96 hours, pulsed with 1 .mu.Ci .sup.3 H thymidine for the last 18 hours and harvested as mentioned above.

#### Detailed Description Text (85):

Because CD40 triggering is known to upregulate a variety of cell surface molecules on B cells, including adhesion molecules (26), it was determined if CD40 ligation upregulates CD54 or CD106 expression on SM fibroblasts. SM fibroblasts were cultured with CD40L.sup.+ Jurkat D1.1 cells in the presence or absence of anti-CD40L mAb 5C8 or control mAb. SM fibroblasts were also cultured with CD40L.sup.+ Jurkat B2.7 cells or CD40L.sup.+ Jurkat B2.7 transfectants. After the indicated period of time in culture, SM fibroblast CD54 or CD106 expression was determined by two-color FACS analysis. CD13 expression was utilized to discriminate SM fibroblasts from Jurkat T cells (27). CD40L.sup.+ D1.1 cells, but not control CD40L.sup.- B2.7 cells, induce a 2-4 fold increase in SM fibroblast CD54 expression (FIGS. 4 and 5) in a manner that is specifically inhibited by mAb 5C8 but not by control mAb (FIG. 4). Moreover, CD40L.sup.+ D1.1 and CD40L.sup.+ Jurkat B2.7 transfectants, but not control CD40L.sup.- B2.7 cells, similarly upregulate SM fibroblast CD106 expression (FIG. 5). Together, these results demonstrate that CD40L-CD40 interactions upregulate SM fibroblast CD54 and CD106 expression.

#### Detailed Description Text (87):

Ligation of CD40 induces B cells (28) and monocytes (12) to produce IL-6. Interestingly, SM fibroblasts produce IL-6 in vivo (29, 30) and in vitro (31). The next series of experiments asked if CD40L-CD40 interactions effect IL-6 secretion by SM fibroblasts. Therefore, SM fibroblasts were cultured with mitomycin-C treated CD40L.sup.+ Jurkat D1.1 cells in the presence or absence of anti-CD40L mAb 5C8 or control mAb. Additionally, SM fibroblasts were cultured with CD40L.sup.- Jurkat B2.7 cells or CD40L.sup.+ Jurkat B2.7 transfectants. Fibroblast supernatants or control supernatants from Jurkat cells cultured alone were collected after 48 hours and dilutions added to the IL-6 responsive murine B cell line B9. D1.1 cells and CD40L B2.7 transfectants, but not CD40L B2.7 cells, augment SM fibroblast IL-6 secretion (FIG. 6). Additionally, anti-CD40L mAb 5C8, but not control mAb, inhibits this effect of D1.1 cells. Control supernatants collected from Jurkat cells cultured alone did not induce B9 proliferation (See description of FIG. 6). These studies indicate that ligation of CD40 on SM fibroblasts augments IL-6 secretion.

#### Detailed Description Text (89):

Because CD40 ligation induces B cell proliferation (5, 21), it was next asked if CD40L.sup.+ cells induce proliferation of SM fibroblasts. Therefore, SM fibroblasts were cultured overnight in 1% FM to arrest growth, as previously described (32), and further additions to the cells were performed in 1% FM, unless otherwise indicated. Mitomycin-C treated CD40L.sup.+ B2.7 transfectants or CD40L.sup.- B2.7 cells were then added to the SM fibroblasts. Where indicated, co-culture experiments also included anti-CD40L mAb 5C8 or isotype control mAb P1.17. In some experiments, SM

fibroblasts were pretreated overnight with rINF- $\gamma$ , prior to the addition of CD40L.sup.+ B2.7 transfectants. Because fibroblasts are known to proliferate in the presence of media containing 10% FCS ((32)), each experiment included control fibroblasts cultured in 10% FM. .sup.3 H thymidine incorporation was determined after 48 hours. CD40L.sup.+ B2.7 transfectants, in contrast to parental CD40L.sup.- B2.7 cells, induce SM fibroblast proliferation (FIG. 7). Furthermore, anti-CD40L mAb 5C8 specifically inhibits the ability of CD40L.sup.+ B2.7 transfectants to induce fibroblast proliferation (FIG. 7). In addition, pretreatment of SM fibroblasts with rINF- $\gamma$  augments the capacity of CD40L.sup.+ B2.7 transfectants to induce SM fibroblast proliferation (FIG. 8). Together, these data demonstrate that CD40L mediated signals induce SM fibroblast proliferation in vitro and this effect is enhanced by rINF- $\gamma$ .

Detailed Description Text (102):

D1.1 is a Jurkat T cell subclone that constitutively expresses CD40L (20, 42). B2.7 is a CD40L.sup.- Jurkat T cell subclone (20, 42). Stably transfected CD40L.sup.+ 293 kidney cells or CD8.sup.+ 293 kidney cells were generated as previously reported (37). Ramos 2G6 B cells respond to CD40L mediated signals (38, 39) and were obtained from ATCC.

Detailed Description Text (108):

To study the effect of CD40 ligation on the expression of HUVEC cell surface molecules, cells were cultured in 6 well plates as described above. When HUVEC were near confluence 1.times.10.sup.6 CD40L.sup.+ Jurkat D1.1 cells, CD40L.sup.- Jurkat B2.7 cells, CD40L.sup.+ 293 kidney cell transfectants or CD8 kidney cell transfectants were added to the culture. Where indicated, CD40L.sup.+ cells were pretreated with anti-CD40L mAb 5C8 (10 .mu.g/ml) or isotype control mAb P1.17 (10 .mu.g/ml) prior to the addition to HUVEC. After the indicated time in culture the cells were collected by trypsinization and two-color FACS analyses performed.

Detailed Description Text (123):

To determine if CD40L mediated signals were sufficient, in the absence of additional lymphoid specific interactions, to upregulate endothelial cell adhesion molecules, HUVEC were cultured with stably transfected CD40L.sup.+ 293 kidney cells or control CD8.sup.+ 293 transfectants. As a positive control, HUVEC were also cultured with CD40L.sup.+ D1.1 cells. Similar to CD40L.sup.+ D1.1 cells, CD40t 293 kidney cell transfectants upregulate CD54, CD62E and CD106 expression on HUVEC (FIG. 15). Control 293 CD8 transfectants have no effect on HUVEC CD54, CD62E or CD106 expression. Together, these studies demonstrate that CD40L-CD40 interactions are sufficient to upregulate these adhesion molecules on HUVEC in vitro.

Detailed Description Text (129):

CD40 is a cell surface molecule constitutively expressed on a variety of cells, including B cells (12, 13), monocytes (14), dendritic cells (15), epithelial cells (17, 18), basophils (16) and fibroblasts (19). The counter-receptor for CD40 is CD40L, a 30-33 kDa activation-induced, transiently expressed CD4.sup.+ T cell surface molecule (20-25). It is shown that endothelial cells in spleen, thyroid, skin, muscle, kidney, lung or umbilical cord express CD40 in situ. This finding is consistent with a previous report that endothelial cells in rheumatoid arthritis synovial membrane express CD40 (11). In addition, human umbilical vein endothelial cells (HUVEC) express CD40 in vitro. Most importantly, CD40 expression on endothelial cells is functionally significant because CD40L.sup.+ Jurkat T cells or CD40L.sup.+ 293 kidney cell transfectants, but not control cells, upregulate the expression of intercellular adhesion molecules CD54 (ICAM-1), CD62E (E-selectin) and CD106 (VCAM-1) on HUVEC. The results disclosed herein demonstrate that endothelial cells express CD40 and CD40L-CD40 interactions induce endothelial cell activation in vitro.



**WEST**

Generate Collection

Print

L1: Entry 47 of 89

File: USPT

Aug 22, 2000

DOCUMENT-IDENTIFIER: US 6106832 A

TITLE: Treatment of individuals exhibiting defective CD40L

**Brief Summary Text (13):**

The present invention also provides a method of utilizing gene therapy to correct X-linked hyper-IgM syndrome and other syndromes in which the CD40L gene does not encode biologically active CD40L. Gene therapy to correct such syndromes comprises isolating CD4+ T cells from an affected individual, transfecting the isolated T cells with a transfection vector that expresses a biologically active CD40L, and administering the transfecting T cells expressing biologically active CD40L to the individual.

**Detailed Description Text (7):**

To ensure that these mutations were not merely naturally occurring gene polymorphisms, two of the nucleotide changes found were introduced separately into a mammalian expression vector containing the complete coding region for the human CD40L using site-directed mutagenesis. Cells were transfected with vectors carrying either the wild type or the mutagenized CD40Ls, metabolically radiolabeled, and examined for expression of CD40L protein by precipitation with a polyclonal serum directed against CV1/EBNA cells expressing the human CD40L, or with CD40.Fc. Cells transfected with the wild type CD40L expressed a 33 kD protein that was detected using CD40.Fc. In contrast, cells transfected with either mutant form of CD40L did not express a protein recognized by CD40.Fc. Immunoprecipitation of identical lysates using the polyclonal antiserum resulted in the recognition of a 33 kD protein, from both mutant and wild type transfected cells, which co-migrated with the CD40L protein recognized by CD40.Fc. Northern blot analysis showed similar levels of CD40L-specific RNA in both wild type and mutant transfected cells. Additionally, cells transfected with either mutant form of CD40L were completely negative for CD40.Fc binding, while cells expressing the wild type CD40L showed strong CD40.Fc binding. Cells expressing either form of mutagenized CD40L were also unable to induce B cell proliferation or IgE secretion, confirming the absence of functional CD40L on their cell surfaces.

**Detailed Description Text (17):**

A gene encoding biologically active CD40L may also be introduced into T cells (preferably CD4+ T cells) obtained from an individual with abnormal or defective CD40L using gene transfer techniques. The cells are isolated, and transfected with the biologically active CD40L gene; they are subsequently re-administered to the individual, and will then correct the symptoms of the syndrome by producing biologically active CD40L.

**Detailed Description Text (39):**

To test whether the nucleotide changes detected in Example 3 affected the expression of the CD40L or its ability to bind to CD40, the two nucleotide changes found in Patient 1 and Patient 2 of Example 2 were introduced separately into a mammalian expression vector containing the complete coding region for the human CD40L using site-directed mutagenesis. A cloning vector containing human CD40L sequence, designated hCD40-L, was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Va. 20110-2209 (ATCC) on Dec. 6, 1991, under accession number 68873. Mutants were constructed using the gene splicing by overlap extension (SOEing) process (Horton et al., BioTechniques 8:528, 1990). The primer used to recreate the mutation found in Patient 1 was 5'-TGCGGGCAACATCCATTCACTTGGGAGTATTGAATTGCAA (SEQ ID NO:5). The primer used to

recreate the mutation found in Patient 2 was 5'-CCATGAGCAACAACCTTGGTAACCCCGGAAAAATGGGAACAGC (SEQ ID NO:6). The remainder of the necessary primers were generated from the CD40L sequence (described in U.S. Ser. No. 07/969,703 and in Armitage et al., Nature 357:80, 1992 and Spriggs et al., J. Exp. Med. 176:1543, 1992). The resultant vectors were referred to as Mutant 1 and Mutant 2, respectively. The introduction of the appropriate nucleotide change was confirmed in the actual expression vectors by sequence analysis of the entire coding region. The human embryonic kidney cell line, 293, was transfected with vectors carrying either the wild type or the mutagenized CD40Ls, and on day 3 post transfection, cells were metabolically radiolabeled with sup.35 S Trans-label (ICN Radiochemicals, Irvine, Calif.). Cell lysates were prepared and examined for expression of CD40L protein by precipitation with a polyclonal serum directed against CV1/EBNA cells expressing the human CD40L, or with CD40.Fc. Cells transfected with the wild type CD40L expressed a 33 kD protein that can be readily precipitated using CD40.Fc. In contrast, cells transfected with either mutant form of CD40L did not express a protein recognized by CD40.Fc. Immunoprecipitation of identical lysates using the polyclonal antiserum, however, resulted in the recognition of a 33 kD protein from mutant as well as wild type transfected cells. This protein co-migrated with the CD40L protein recognized by the CD40.Fc and was not present in lysates transfected with vector alone. Consistent with these results, Northern blot analysis showed similar levels of CD40L-specific RNA in both wild type and mutant transfected cells.

#### Detailed Description Text (40):

Transfected cells were also examined by flow cytometric analysis. Cells transfected with either mutant form of CD40L were completely negative for CD40.Fc binding, while cells expressing the wild type CD40L showed strong CD40.Fc binding. To address the biological activity of the mutant CD40L proteins, cells transfected with wild type or mutagenized CD40Ls were examined for their ability to induce proliferation and IgE secretion from purified tonsil B cells co-cultured with IL-4. In contrast to cells transfected with wild type ligand (Table 1), cells expressing either form of mutagenized CD40L were unable to induce B cell proliferation or IgE secretion, confirming the absence of functional CD40L on their cell surface.

#### Detailed Description Text (44):

Previous work has shown that culture of single donor PBMC in the presence of IL-4 results in the production of IgE (IgE secretion from 1.times.10.sup.5 unfractionated to T-depleted PBMC was determined following 10 days culture with 5 ng/ml IL-4, together with 200 ng/ml G28-5 antibody (monoclonal antibody to CD40, obtained from Dr. E. A. Clark, University of Washington, Seattle, Wash.) or 1.times.10.sup.4 fixed CV1/EBNA cells transfected with vector alone or human CD40L. Preparation of PBMC and determination of secreted IgE concentrations were performed as described in Fanslow et al., J. Immunol. 149:655, 1992. Results are expressed as the mean +/- SEM of triplicate cultures. PBMC from normal donors (Controls 1, 3, and 4) produced measurable amounts of IgE when cultured with IL-4 (Table 2). In contrast, no IgE production was detected from any of the four hyper-IgM patients cultured under the same conditions. Significantly, in 3 out of 4 cases (Patients 1, 2, and 4), the addition of recombinant CD40L or the CD40 mAb, G28-5, to cultures containing hyper-IgM patients' PBMC restored their ability to secrete IgE. Similarly, the T-depleted PBMC (B-cell enriched cultures) from these three patients and from all controls examined secreted IgE in the presence of IL-4 plus either recombinant CD40L or G28-5 antibody (Table 2). In the case of Patient 3, no IgE was detected in PBMC cultured with IL-4 and recombinant CD40L or G28-5 antibody. The reason for these results is unclear. Additional PBMC were not available from this patient; thus, it was not possible to determine whether this lack of response was reproducible or due to experimental variation.

#### Detailed Description Paragraph Table (1):

TABLE 1	Mutagenized CD40 ligands are not biologically active	Number of <u>Transfected</u> Cells	Vector alone	Wild type	Mutant 1	Mutant 2
6463	+. .	911 375	+. .	122 407	+. .	92 IgE.dagger.

#### Detailed Description Paragraph Table (3):

TABLE 2	PBMC from hyper-IgM patients can
---------	----------------------------------

secrete IgE IgE Secreted\* Patient/Control 1 2 3 4

A. Unfractionated PBMC Vector alone Patient  
 <0.3 <0.3 <0.3 <0.3 Control 8.6 .+- . 1.2 <0.3 16.3 .+- . 2.9 22.9 .+- . 3.7 CD40  
ligand Patient 43.3 .+- . 4.8 51.0 .+- . 12.4 <0.3 33.5 .+- . 6.0 Control 73.4 .+- . 6.7  
 <0.3 48.1 .+- . 4.4 25.7 .+- . 4.0 G28-5 mAb Patient 76.4 .+- . 6.8 54.7 .+- . 12.5 <0.3  
 55.0 .+- . 8.6 Control 87.1 .+- . 9.8 <0.3 87.2 .+- . 5.8 84.4 .+- . 8.2 B. T-depleted  
 PBMC Vector alone Patient <0.3 <0.3 <0.3 <0.3 Control <0.3 <0.3 <0.3 <0.3 CD40  
ligand Patient 39.2 .+- . 4.4 22.6 .+- . 3.1 <0.3 23.1 .+- . 6.3 Control 128.4 .+- .  
 13.6 <0.3 55.1 .+- . 4.6 80.2 .+- . 8.3 G28-5 mAb Patient 69.4 .+- . 6.2 34.4 .+- . 11.9  
 <0.3 45.5 .+- . 6.0 Control 146.0 .+- . 12.2 <0.3 93.5 .+- . 6.1 98.0 .+- . 9.5

\*IgE secretion from 1 .times. 10.sup.5  
 purified tonsil B cells was measured after 10 days coculture with 5 ng/ml IL4,  
 together with 200 ng/ml G285 antibody or with CV1/EBNA cells transfected with CD40L  
 or vector alone.

**WEST**

Generate Collection

Print

L1: Entry 66 of 89

File: USPT

Aug 31, 1999

DOCUMENT-IDENTIFIER: US 5945513 A

TITLE: Fusion proteins comprising gp39 and CD8

**Brief Summary Text (9):**

Recently, a cDNA encoding murine gp39 has been isolated and shown to be functionally active when expressed as a membrane protein on transfected cells (Armitage et al., 1992, Nature 357:80-82). This cDNA encodes a 260 amino acid polypeptide with the typical features of a type II membrane protein and CV1/EBNA cells expressing murine gp39 were shown to induce murine and human B cell proliferation without additional co-stimulus.

**Drawing Description Text (3):**

FIG. 2. Soluble recombinant human gp39 and CD72, sgp39 and sCD72. (A) The cDNA fragment encoding the extracellular domain of murine CD8 is designated mu-CD8 EC. The murine CD8 amino terminal secretory signal sequence is shown stippled. The cDNA fragment encoding the extracellular domain of human gp39 or CD72 are designated hu-gp39 EC and hu-CD72 EC, respectively. The amino acid sequences predicted at the site of fusion of the extracellular domain of murine CD8 and human gp39 (italic) or CD72 (italic) are shown below the individual diagrams. Residues introduced at the junction of the two cDNA fragments are shown underlined. The unique Bam HI restriction enzyme recognition site at the junction of the two genes is shown. (B) Radiolabelled proteins from the supernatants of metabolically labeled mock (lanes 1 and 2) of CD8-gp39 (lanes 3 and 4) transfected COS cells were immunoprecipitated based on their interaction with the anti-murine CD8 mAb 53-6 (lanes 1 and 3) or the CD40-Ig (lanes 2 and 4) and analyzed by SDS-PAGE under reducing conditions as described in the text. The electrophoretic mobility of molecular mass standards of the indicated mass in kDa are shown to the left. (C) Radiolabelled proteins from the supernatants of metabolically labeled mock (lanes 1-4) and CD8-CD72 (lanes 5-8) transfected COS cells were recovered based on their reactivity with the anti-murine mAb 53.6 (lanes 1 and 5), the anti-CD72 mAb J3101 (lanes 2 and 6), the anti-CD72 mAb BU41 (lanes 3 and 7) and CD40-Ig (lanes 4 and 8) and analyzed by SDS-PAGE under reducing conditions as described in the text. The electrophoretic mobility of molecular mass standards of the indicated mass in kDa are shown to the left.

**Drawing Description Text (4):**

FIG. 3. Binding of sgp39 or CD40-Ig to transfected COS cells. COS cells transfected with either a gp39 (A and B) or a CD40 (C-F) cDNA expression plasmid were examined for their ability to bind either soluble recombinant CD40 (A and B), or soluble recombinant gp39 (C and D), or the anti-CD40 mAb G28-5 (E and F) as described in the text. Phase (A, C and E) and fluorescent (B, D and F) images of representative fields are shown.

**Drawing Description Text (6):**

FIG. 5. Activation of human B cells by surface bound gp39. The ability of gp39-expressing COS cells (gp39-COS) or mock transfected COS cells (mock COS) to stimulate the proliferation of resting human peripheral blood B cells alone or in the presence of the anti-CD20 mAb IF5 (+IF5) or PMA (+PMA) in the absence (solid bars, alone) or presence (hatched bars, +CD40-Ig) of CD40-Ig was examined as described in the text and evaluated by [<sup>3</sup>H]-thymidine incorporation.

**Detailed Description Text (12):**

In a specific, non-limiting embodiment of the invention, cDNA encoding hgp39 may be isolated and characterized as follows. CD40-Ig, as described in Noelle et al., 1992,

Proc. Natl. Acad. Sci. U.S.A. 89:6550-6554, may be modified by the introduction of three mutations, namely L234F, L235E, and G237A, in the immunoglobulin domain, which reduce the binding to Fc receptors. The modified CD40-Ig may be purified from COS cell supernatants as described in Aruffo, 1990, Cell 61:1303-1313. Human gp39 cDNA may be amplified by polymerase chain reaction (PCR) from a library prepared from phytohemagglutinin-activated human peripheral blood T-cells (Camerini et al., 1989, Nature 342:78-82). The oligonucleotide primers may be designed based on the sequence of murine gp39 (Armitage et al., 1992, Nature 357:80-82) and may be engineered to include cleavage sites for the restriction enzymes XbaI and HindIII, to be used in subcloning the PCR product. For example, and not by way of limitation, the following oligonucleotides may be used: 5'-GCG AAG CTT TCA GTC AGC ATG ATA GAA ACA-3' SEQUENCE ID NO. 15 and 5'-CGC TCT AGA TGT TCA GAG TTT GAG TAA GCC-3' SEQUENCE ID NO. 14. Amplification may be performed with Taq polymerase and the reaction buffer recommended by the manufacturer (Perkin Elmer Cetus Corp., Norwalk, Conn.) using 30 cycles of the following temperature program: 2 min., 95.degree. C.; 2 min., 55.degree. C.; 3 min., 72.degree. C. The PCR product may be digested with HindIII and XbaI and should be found to contain an internal HindIII restriction site. The resulting HindIII-XbaI fragment may then be subcloned into a suitable vector, such as, for example, the CDM8 vector. The complete gene product may be constructed by subcloning the HindIII-HindIII fragment into the vector containing the HindIII-XbaI fragment. The resulting construct may then be transfected into COS cells using DEAE-dextran as described in Aruffo et al., 1990, Cell 61:1303-1313. Transfectants may be stained with CD40-Ig (25 .mu.g/ml in DMEM media) followed by FITC-conjugated goat anti-human IgG Fc antibody (1:50 dilution in DMEM, TAGO, Burlingame, Calif.) and visualized by immunofluorescence microscopy. A clone containing the complete hgp39 sequence may be obtained by colony hybridization as described in Sambrook et al., 1989, in "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Press, Cold Spring Harbor, NY. The subcloned HindIII-HindIII fragment of the PCR product may be used to generate a .sup.32 P-labelled probe by random primed polymerization. Plasmid DNA from several individual clones may be transfected into COS cells and the transfectants may be stained with CD40-Ig. Clones that give rise to positive-staining COS cell transfectants may then be further characterized by restriction fragment mapping and sequencing.

#### Detailed Description Text (50):

CD40-Ig, as described in Noelle et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89:6550-6554, was modified by the introduction of three mutations, namely L234F, L235E, and G237A, in the immunoglobulin domain to reduce the binding to Fc receptors. The modified CD40-Ig was purified from COS cell supernatants as previously described (Aruffo et al., 1990, Cell 61:1303-1313). Human gp39 cDNA was amplified by polymerase chain reaction (PCR) from a library prepared from mRNA isolated from PHA-activated human peripheral blood T-cells (Camerini et al., 1989, Nature 342:78-82). The oligonucleotide primers were designed based on the sequence of the murine gp39 (Armitage et al., 1992, Nature, 357:80-82) and included sites for the restriction enzymes Xba I and HindIII to be used in subcloning the PCR product. The oligonucleotides used were: 5'-GCG AAG CTT TCA GTC AGC ATG ATA GAA ACA-3' SEQUENCE ID NO. 13 and 5'-CGC TCT AGA TGT TCA GAG TTT GAG TAA GCC-3' SEQUENCE ID NO. 14. Amplification was performed with Taq polymerase and the reaction buffer recommended by the manufacturer (Perkin Elmer Cetus Corp., Norwalk, Conn.) using 30 cycles of the following temperature program: 2 min., 95.degree. C.; 2 min., 55.degree. C.; 3 min., 72.degree. C. The PCR product was digested with HindIII and XbaI and was found to contain an internal HindIII restriction site. The HindIII-XbaI fragment was subcloned into the CDM8 vector. The complete gene product was constructed by subcloning the HindIII-HindIII fragment into the vector containing the HindIII-XbaI fragment. The resulting construct was transfected into COS cells using DEAE-dextran as described in Aruffo et al., 1990, Cell 61:1303-1313). Transfectants were stained with CD40-Ig (25 .mu.g/ml in DMEM media) followed by FITC-conjugated goat anti-human IgG Fc antibody (1:50 dilution in DMEM, TAGO, Burlingame, Calif.) and visualized by immunofluorescence microscopy. The complete human gp39 was obtained by colony hybridization as described (Sambrook et al., 1989, "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The subcloned HindIII-HindIII fragment of the PCR product was used to generate a .sup.32 P-labeled probe by random primed polymerization. Plasmid DNA from three individual clones were transfected into COS cells and cells were stained with CD40-Ig. One clone, clone 19, was positive by this criteria and was

used in the remainder of the study. The sequence was determined by dideoxy sequencing using Sequenase.TM. (United States Biochemical Co., Cleveland, Ohio)

Detailed Description Text (52):

A cDNA encoding the human gp39 was amplified from a cDNA library prepared from mRNA isolated from PHA activated human peripheral blood T cells by the polymerase chain reaction (PCR) using synthetic oligonucleotides based on the murine gp39 sequence (Armitage et al., 1992, Nature 357:80-82). The PCR product was subcloned into the expression vector CDM8 (Seed, 1987, Nature 329:840-842). COS cells transfected with the CDM8-gp39 plasmid produced protein which bound to CD40-Ig (Noelle et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89:6550-6554). A complete human gp39 gene was isolated by colony hybridization from the same cDNA library that was used for the PCR amplification of gp39 using the subcloned PCR product as a probe. A number of positive clones were isolated and analyzed by restriction enzyme digestion. DNA corresponding to those clones containing the largest inserts, 1.8-1.5 kb, were transfected into COS cells and their ability to direct the expression of a CD40-Ig binding protein examined. One such clone was positive by this criteria and was analyzed further and is referred to hereafter as human gp39. Immunoprecipitation of cDNA-encoded human gp39 protein from transfected COS cells using CD40-Ig showed a single band corresponding to a molecular mass of about 32-33 kDa. The COS-cell derived protein is smaller than we had expected based on our previous studies of murine gp39, however, we have observed in many instances that the apparent molecular masses of a number of different T cell surface proteins obtained from COS cell transfectants are smaller than those obtained from T cells (Aruffo and Seed, 1987, EMBO J. 11:3313-3316; Aruffo et al., 1991, J. Exp. Med. 174:949-952). These differences in size may be the result of incomplete glycosylation of the proteins by COS cells.

Detailed Description Text (66):

The binding of hgp39 and CD40 to the soluble forms of their respective ligands was tested by staining of transfected COS cells. COS cells were transfected with CD40, hgp39 or vector alone (mock) using DEAE-dextran. One day after transfection, cells were trypsinized and replated. Cells were stained on the following day. Cells expressing gp39 or mock transfected cells were stained with CD40-Ig (25 µg/ml) followed by FITC-conjugated goat anti-human Fc. Cells expressing CD40 were stained by incubation with COS cell supernatants containing shgp39 followed by mAb 53-6 (anti-murine CD8, 2.5 µg/ml) then FITC-conjugated goat anti-rat Fc (Organon Teknika Co., West Chester, Pa., 1.5 µg/ml). As controls, COS cells expressing CD40 were stained with FITC-conjugated G28-5 (anti-CD40) or using COS cell supernatants containing sCD72. All incubations were done at room temperature in PBS containing 1 mM CaCl<sub>2</sub>.sub.2, 1 mM MgCl<sub>2</sub>.sub.2 and 2% FBS and the same buffer was used for all washes. Following staining, cells were fixed with 1% paraformaldehyde in PBS.

Detailed Description Text (71):

COS cells transfected with the gp39 construct or vector alone (mock-COS) were harvested from tissue culture plates with EDTA, washed twice with PBS, suspended at 5.times.10.sup.6 cells/ml and irradiated with 5000 rads from a 137 Cs source. COS cells were used at a ratio of 1:4 (1.times.10.sup.4 COS cells vs. 4.times.10.sup.4 B cells) in proliferation assays.

Detailed Description Text (77):

To further characterize the interaction between CD40 and the soluble recombinant hgp39, COS cells were transfected with a cDNA encoding the full length CD40 protein (Stamenkovic et al. 1989, EMBO J. 8:1403-1410) and their ability to bind to shgp39, sCD72, and anti-CD40 mAb examined by fluorescence microscopy. Both the shgp39 and the anti-CD40 mAb bound to the transfectants while sCD72 did not (FIG. 3). In addition, COS cells were transfected with a cDNA encoding the surface bound gp39 and their ability to bind to CD40-Ig (Noelle et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89:6550-6554) or an irrelevant Ig fusion protein, Leu8-Ig (Aruffo et al. 1992, Proc. Natl. Acad. Sci. U.S.A. 89:2292-2296), examined. CD40-Ig, but not Leu8-Ig, bound to gp39 expressing COS cells (FIG. 3). In parallel experiments, shgp39 and CD72 were immobilized in the wells of a 96 well microtiter dish via an anti-CD8 mAb and their binding to increasing concentrations of CD40-Ig or a control immunoglobulin fusion protein, Leu8-Ig, examined. The binding of CD40-Ig to

immobilized shgp39 was saturable, while CD40-Ig did not bind to sCD72 and Leu8-Ig did not bind to shgp39 (FIG. 4).

Detailed Description Text (79):

To examine the role of gp39-CD40 interactions in B cell activation, COS cells transfected with either the cDNA encoding hgp39 or vector alone (mock) were tested for their ability to stimulate B cell proliferation. Resting, peripheral blood B cells proliferated only weakly when incubated with hgp39-expressing COS cells alone (FIG. 5). However, upon exposure to hgp39-expressing COS cells in conjunction with either (i) 1F5 mAb (Clark et al., 1985, Proc. Natl. Acad. Sci. U.S.A. 82:1766-1770), directed against the B cell surface protein CD20, or (ii) PMA, vigorous B cell proliferation was observed. In both cases, the hgp39driven B cell proliferation could be reduced to background levels with the soluble CD40-Ig fusion protein (FIG. 5). B cells proliferated weakly when incubated with mock transfected COS cells in the presence of either the anti-CD20 mAb or PMA and this proliferation was unaffected by the presence of CD40-Ig (FIG. 5). The weak B cell proliferation observed with hgp39-expressing COS cells in the absence of a co-stimulatory signal suggests that in this case COS cells also provide co-stimulatory signals that synergize with CD40 signals to drive B cell proliferation.

**WEST**

Generate Collection

Print

L1: Entry 75 of 89

File: USPT

Nov 17, 1998

DOCUMENT-IDENTIFIER: US 5837816 A

TITLE: Interleukin-2 receptor subunit ectodomain fusion protein comprising a leucine zipper domain

Detailed Description Text (2):

The present invention relates to a method of preparing a soluble hetero-oligomeric mammalian polypeptide (or protein) by culturing a host cell transformed or transfected with an expression vector encoding a fusion protein comprising a leucine zipper domain and a heterologous mammalian protein. Preferably, the heterologous mammalian protein comprises subunit proteins that function cooperatively to bind the ligand. In one embodiment, the heterologous mammalian protein comprises the IL-2 alpha and beta receptor ectodomains. Cooperative binding can occur with different receptors that act together to bind a ligand (such as IL-2), for example. Exemplary mammalian transmembrane proteins include members of the tumor necrosis factor/nerve growth factor receptor (TNFR/NGFR) family (Farrah and Smith, Nature 358:26, 1992; Goodwin et al., Cell 73:447; 1993), which includes CD40 Ligand (CD40-L), CD27 Ligand (CD27-L), OX40 Ligand (OX40-L), and TNF. Structural studies of certain members of this family of proteins indicate that they form homotrimers. The inventive method will also be useful for other members of this family.



**WEST**

Generate Collection

Print

L1: Entry 85 of 89

File: USPT

Oct 7, 1997

DOCUMENT-IDENTIFIER: US 5674492 A

TITLE: Method of preventing or treating disease characterized by neoplastic cells expressing CD40

Detailed Description Text (21):

The CD40L/FC2 and the trimeric CD40L described in U.S.S.N. 07/969,703 will be useful in the present inventive methods, as will other forms of CD40L that can be prepared using known methods of preparing recombinant proteins. Such recombinant proteins include novel polypeptides that can act as a ligand for murine and human CD40, which have been isolated and sequenced. More particularly, cDNAs encoding these ligands have been cloned and sequenced as described in USSN 07/969,703, filed Oct. 23, 1992. Further provided therein are methods for expression of recombinant CD40L polypeptides. CD40L polypeptide include other forms of mammalian CD40L, such as derivatives or analogs of human or murine CD40L. Murine and human CD40L comprise a 214 and 215, respectively amino acid extracellular region at the C-terminus of full length, membrane-bound polypeptide. The extracellular region contains the domain that binds to CD40. Murine and human CD40L further comprise a homologous hydrophobic 24 amino acid transmembrane region delineated by charged amino acids on either side and a 22 amino acid intracellular region at their N-termini. The present invention further comprises full length CD40L polypeptides or fragments thereof comprising all or part of the extracellular region or derivatives of the extracellular region and mammalian cells transfected with a cDNA encoding murine or human CD40L and expressing human or murine CD40 L as a membrane-bound protein.

Detailed Description Text (22):

The present invention comprises isolated DNA sequences encoding CD40L polypeptides and DNA or RNA sequences complementary to such isolated DNA sequences. The isolated DNA sequences and their complements are selected from the group consisting of (a) nucleotides 184 through 828, 193 through 828, 193 through 762, or 403 through 762 of the DNA sequence set forth in SEQ ID NO:1 and their complements, (b) DNA sequences which hybridize to the DNA sequences of (a) or their complements under conditions of moderate stringency and which encode a CD40L polypeptide, analogs or derivatives thereof, and (c) DNA sequences which, due to the degeneracy of the genetic code, encode CD40L polypeptides encoded by any of the foregoing DNA sequences and their complements. In addition, the present invention includes vectors comprising DNA sequences encoding CD40L polypeptides and analogs, and host cells transfected with such vectors

Detailed Description Text (63):

This example illustrates the effect of CD40 ligand on the growth of B-cell lymphomas in vitro. Soluble CD40 ligand (CD40L; described in U.S.S.N. 08/477,733, and U.S.S.N. 08/484,624, both of which are continuations in part of U.S.S.N. 08/249,189, which is a continuation in part of U.S.S.N. 07/969,703, now abandoned) was obtained from transfected COS-7 cells as supernatant fluid, and tested in a proliferation assay used as described above, in Example 2, using RL or TU2C cells. Both murine and human CD40L-containing supernatant fluids were tested, since murine CD40L binds to human cells that express CD40, and acts as a costimulus in the same manner as human CD40L. Each lot of supernatant fluid was titrated to determine the concentration that yielded optimal inhibition of proliferation; a 1:5 dilution yielded maximal inhibition.

Detailed Description Text (64):

Exemplary results are presented in FIG. 4; values are presented as percent of

inhibition compared to control supernatant fluids. The soluble human ligand was inhibitory for the various lymphomas tested, with maximal inhibition seen (50-80%) on RL and TU2C cell lines at a 1:5 dilution of the supernatant fluid. The soluble murine CD40L produced similar, if not better, inhibitory effects. Control supernatant fluid from COS-7 cells transfected with vector alone actually promoted lymphoma cell growth. Accordingly, the inhibitory effects of CD40L on B lymphomas parallels that of antibodies to CD40.

Detailed Description Text (88):

This example illustrates the effect of recombinant human CD40 ligand on the growth of human B-cell lymphomas in SCID mice. SCID mice were obtained, and treated substantially as described in Example 4, above. On day 0, SCID mice were injected either intraperitoneally with 5.times.10<sup>6</sup> RL or TU2C cells. The tumor cell recipients then received 100 .mu.l of concentrated supernatant fluid from cells transfected with either a vector encoding human CD40 ligand, or vector alone (control). Two concentrations of the CD40 ligand-containing supernatant fluid were tested: a ten-fold concentrate and a two-fold concentrate (10x and 2x, respectively). The concentrated supernatants were administered intraperitoneally every third day for a period of 15 days (total of 5 injections), starting at day 3. Mice were monitored for tumor development and progression; moribund mice were euthanized. All mice were necropsied for evidence of tumor. Liver, kidney and lymphoid organs were analyzed histologically for presence of tumor cells. Both parametric (student's t test) and non-parametric (Wilcoxon rank sum test) analyses were performed to determine if the groups differed significantly (p<0.05). All experiments had 7-10 mice per group, and were performed 3 times.

**WEST**

Generate Collection

Print

L1: Entry 86 of 89

File: USPT

Jan 21, 1997

DOCUMENT-IDENTIFIER: US 5596072 A  
TITLE: Method of refolding human IL-13

**Drawing Description Text (13):**

FIGS. 12A-12D show aggregation of human B cells induced by CD40L transfectants. JY cells (2.times.10.sup.5) were co-cultured for 5 h in 1 ml with 10.sup.4 COS-7 cells transfected with pJFE14 vector (FIG. 12A) or transfected with pJFE-14-hCD40L and expressing human CD40L (FIG. 12B). Similarly, CD20.sup.+ (>98%) B cells (5,000) were co-cultured for 24 h with 500 COS-7 cells either not expressing (FIG. 12C) or expressing (FIG. 12D) the human CD40L.

**Detailed Description Text (269):**

COS-7 cells transfected with hCD40L (COS-7/hCD40L) induced human B cell activation as judged by the induction of homotypic aggregates of Epstein-Barr Virus (EBV) transformed, and normal B cells. In addition, COS-7/hCD40L induced B cell proliferation, which was further enhanced by IL-4, or IL-13. IL-13, like IL-4, synergized with the mouse- and hCD40L to induce IgM, total IgG, IgG4, and IgE, but not IgA production by highly purified B cells. Anti-IL-4 antibodies inhibited IL-4 and COS-7/hCD40L induced Ig production by B cells, but had no effect on IL-13 and COS-7/hCD40L induced B cell differentiation, indicating that IL-13 and hCD40L induced Ig production, including isotype switching to IgE, independently of IL-4. hCD40L induced B cell differentiation was blocked by soluble CD40, confirming the requirement for specific engagement of CD40L. Collectively, these data indicate that CD40L and IL-13 expressed by human CD4.sup.+ T helper cells are important components of T and B cell interactions resulting in B cell proliferation, differentiation, and IgE switching. However, the distribution of the hCD40L suggests a broader function of this molecule.

**Detailed Description Text (271):**

The contact mediated signals delivered by activated T helper cells can be replaced by anti-CD40 mAbs. One of the contact T helper signals is delivered by the CD40 ligand (CD40L), a 33 kDa molecule expressed on activated CD4.sup.+ T cells. CD40L transfectants induced proliferation of B cells and induced IgE production in the presence of IL-4. Here is described the isolation of human CD40L clones from a cDNA library constructed from an activated CD8.sup.+ T-cell clone. The distribution of the human CD40L, its ability to activate B cells, and its role as a co-activation molecule with IL-13 compared with IL-4 to differentiate B cells were assessed. Cells transfected with the human CD40L exhibited induced B cell aggregation, proliferation, and considerable Ig production, including IgE synthesis, in the presence of IL-13.

**Detailed Description Text (275):**

B lymphocytes (>98% CD20+) were purified from spleen using density gradient centrifugation over Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N.J.), followed by negative cell sorting using a FACStar Plus (Becton Dickinson). Surface Ig $\alpha$  positive cells were sorted directly from the negatively sorted B cell population. The CD4.sup.+ T-cell clone B21 and the CD8.sup.+ T-cell clone A10 have been described by Roncarolo et al. (1988) J. Exp. Med. 167:1523-1534. In co-culture experiments, various numbers of purified B cells were cultured with different concentrations of COS-7 cells in U-bottom 96-well trays in 0.2 ml as indicated in the text. After 10 days, 50% of the medium was replenished, and after 14 days the supernatants harvested and assayed for Ig's by ELISA. COS-7 cells were transiently transfected. For CD40L staining, COS-7 or B21 cells were incubated on ice with 1.4

.mu.g/ml biotinylated CD40-Ig in PBS, 1% FCS for 20 min, washed twice in PBS with 1% FCS, and stained with 1/5 dilution of streptavidin-PE, and washed twice again. Cells specifically expressing CD40L were sorted using a FACStar Plus (Becton Dickinson) before use.

**Detailed Description Text (283):**

Using a biotinylated human CD40-Fc fusion protein in combination with streptavidin-PE, specific expression could be easily detected of human CD40L on COS-7 cells transiently transfected with an expression plasmid pJFE14 containing the 2.1 kb human CD40L cDNA, but not on control cells transfected with empty pJFE14 vector DNA. The human CD40-Fc reagent reacted also with COS-7 cells transfected with the same expression vector containing the mouse CD40L cDNA, which is consistent with previous studies, indicating cross-species binding of human CD40 to mouse CD40L.

**Detailed Description Text (285):**

B cell activation with antibodies to CD40 results in homotypic aggregation. To determine whether the CD40L had similar effects, COS-7 cells expressing the CD40L were purified by FACS and co-cultured with purified B cells or JY cells, an EBV transformed B cell line. Indeed, aggregation of JY cells following incubation with the COS-7 cells expressing human or mouse CD40L was observed, whereas mock-transfected COS-7 cells were ineffective (see FIGS. 12A through 12D). Similarly, purified B cells co-cultured with cells expressing human or mouse CD40L displayed marked homotypic aggregations, whereas B cells cultured with untransfected COS-7 cells remained disperse (FIGS. 12A through 12D).

**Detailed Description Text (288):**

COS-7 cells expressing human or mouse CD40L also induced Ig production by highly purified naive surface IgD+ human B cells in the presence of IL-4 or IL-13 (Table 7). Considerable levels of IgM, IgG4, total IgG and IgE, but no IgA were produced. There was no IgA production is compatible with previous observations which indicated that IL-4 specifically inhibits IgA synthesis under these culture conditions (9). Ig levels induced by IL-13 were in the same range as those induced by IL-4. No Ig production was obtained in the presence of mock-transfected COS-7 cells (Table 7). Induction of all Ig isotypes by COS-7 cells expressing CD40L was effectively blocked by CD40-Ig (10 .mu.g/ml), confirming that specific engagement of the CD40L is necessary for induction of B cell differentiation and Ig production. Inhibition of total IgG production by CD40Ig could not be measured, since the Ig portion of the CD40-Ig fusion protein gave a strong signal in the IgG ELISA. Interestingly, Ig production, including IgG4 and IgE production, induced by IL-13 in the presence of COS-7/CD40L cells was not blocked by anti-IL-4 mAbs (10 .mu.g/ml), whereas these mAbs strongly blocked IL-4-induced Ig production in the presence of COS-7/CD40L (Table 7). These results indicate that IL-13 induces Ig production independently from IL-4. These data furthermore indicate that IL-13 is another cytokine that directs naive surface IgD+ human B cells to switch to IgG4 and IgE producing cells in the presence of a contact-mediated costimulatory signal delivered by COS-7 cells expressing the mouse or human CD40L.

**Detailed Description Text (291):**

In the present study it is demonstrated that the human CD40L cDNA, which was cloned and expressed in COS7 cells is very effective in inducing human B cell activation. COS7/hCD40L induced homotypic aggregation of EBV-transformed and normal B cells and B cell proliferation, similarly as observed with anti-CD40 mAbs. In addition, differentiation of B cells into Ig secreting plasma cells was observed in the presence of IL-4 or IL-13. The 2.1 kb hCD40L cDNA was isolated from a CD8.sup.+ T cell cDNA library and appeared to be a full-length clone, which was by sequence comparison, identical to the 1.8 kb cDNA's described earlier. An additional 1.2 kb cDNA clone probably represents a second mRNA species of that size which was detected in activated T cells and which apparently encodes the same protein. The hCD40L has 80% homology with the corresponding mouse gene. Interestingly, the hCD40L has also some degree of homology with TNF-.alpha. and TNF-.beta.. The positioning of the four cysteine residues and the potential extracellular N-linked glycosylation site in the mouse CD40L are conserved in the human CD40L, however the human protein has an additional cysteine substituted at position 194. The CD40L is reported to be a type II membrane anchored protein and there is a hydrophobic region of the human protein (amino acids 22-45) representing a potential signal/anchor domain near the amino

terminus. B cell proliferation induced by COS7/CD40L was enhanced by IL-4 or IL-13. IL-4 and IL-13 seemed to be equally effective, indicating that IL-13, like IL-4, has B cell growth promoting activity. IL-13, like IL-4, also induced Ig production in cultures of naive surface IgD.sup.+ B cells that have been co-stimulated by COS7/hCD40L. Considerable levels of IgM, IgG4, total IgG, and IgE were produced under these culture conditions. The profile of Ig production induced by IL-4 and IL-13 with hCD40L is similar to that obtained in the presence of IL-4 and anti-CD40 mAbs. Thus IL-13 and IL-4 appear equally potent in inducing both proliferation and Ig synthesis in B cells. Furthermore, these results indicate that the hCD40L provides a co-stimulatory signal for IL-4 or IL-13 induced B cell differentiation, confirming the important role for CD40 in B cell activation and differentiation. Since these experiments were carried out with naive sigD.sup.+, these results confirm previous observations that IL-13, in addition to IL-4, is another CD4.sup.+ T cell derived lymphokine that can direct B cells to switch to IgG4 and IgE producing cells (see Table B1 and FIGS. 5A through 5E). Ig production, including IgG4 and IgE production, induced by IL-13 in the presence of hCD40L was not blocked by anti-IL-4 mAbs, indicating that the effects of IL-13 are mediated independently of IL-4.

#### Detailed Description Text (292):

Help provided by the CD40L transfectants, and the specific blocking of this help by CD40-Ig, indicates that expression of CD40L on CD4.sup.+ T cells may be an important component of both antigenic and non-specific T-B cell interactions, leading to B cell activation and differentiation. These data are compatible with blocking studies carried out with mAbs against mouse CD40L, or CD40-Ig, which indicated that CD40L and CD40 interaction is critical for T cell help in the mouse system. It is of importance to note that there is a difference in the consequences of signaling by CD40 and activated CD4.sup.+ T cells suggesting that additional T cell surface molecules may be involved in productive T-B cell interaction. In fact, the transmembrane form of TNF.alpha. expressed on activated CD4.sup.+ T cells is also associated with T cell induced B cell activation and differentiation.

#### Detailed Description Paragraph Table (6):

TABLE 6 Induction of B cell proliferation by IL-13 or IL-4 and COS cells expressing the human or mouse CD40-L .sup.3 H TdR incorporation (c.p.m. .times. 10.sup.-3)

	B
0.1 .+-. 0 B + IL-13 0.1 .+-. 0 B + IL-4 0.2 .+-. 0 B + IL-4 + control mAb 0.2 .+-. 0 B + IL-4 + anti-CD40 21.2 .+-. 4.1 COS hCD40-L 1.1 .+-. 0.2 COS mCD40-L 1.0 .+-. 0.1 COS 1.4 .+-. 0.2 B + COS hCD40-L 16.9 .+-. 2.4 B + COS mCD40-L 17.5 .+-. 2.1 B + COS 1.2 .+-. 0.2 B + IL-4 COS hCD40-L 30.7 .+-. 3.8 B + IL-4 COS mCD40-L 35.4 .+-. 4.5 B + IL-4 COS 1.3 .+-. 0.2 B + IL-13 COS hCD40-L 22.5 .+-. 3.0 B + IL-13 COS mCD40-L 33.8 .+-. 2.8 B + IL-13 COS 1.2 .+-. 0.4	

Five .times. 10.sup.4 highly purified (>98% CD20.sup.+) negatively sorted splenic B cells were cocultured with 1.6 .times. 10.sup.4 irradiated (7,000 rads) COS cells transfected with human or mouse CD40L or the empty pJFE14 vector as control. IL13 or IL4 were added at 400 U/ml. Soluble antiCD40 mAb 89 and the control mAb A4 were used at 50 .mu.g/ml. The cultures were harvested 3 days later after addition of .sup.3 H Thymidine in the last 16 hours of culture. The values represent means and standard deviations of triplicate cultures.

#### Detailed Description Paragraph Table (7):

TABLE 7 Induction of Ig synthesis by IL-13 or IL-4 and COS cells expressing CD40-L IgM IgG IgG4 IgE ng/ml

	COS
hCD40-L 4 .+-. 2 38 .+-. 4 12 .+-. 1 <0.2 COS mCD40-L <0.2 6 .+-. 0 3 .+-. 0 <0.2 IL-4 + COS hCD40-L 87 .+-. 8 195 .+-. 21 148 .+-. 30 80 .+-. 4 IL-4 + COS hCD40-L + CD40-Ig 3 .+-. 0.5 ND* 1.8 .+-. 1.4 2.7 .+-. 1.2 IL-4 + COS hCD40-L + anti-IL-4 8 .+-. 3 28 .+-. 7 5 .+-. 3 4 .+-. 2 IL-4 + COS mCD40-L 64 .+-. 6 208 .+-. 5 177 .+-. 42 68 .+-. 7 IL-4 + COS mCD40-L + CD40-Ig <0.2 ND* <0.4 <0.2 IL-4 + COS 0 .+-. 0 4 .+-. 0 1 .+-. 0 <0.2 IL-13 + COS hCD40-L 51 .+-. 1 151 .+-. 9 127 .+-. 9 54 .+-. 7 IL-13 + COS mCD40-L 31 .+-. 3 100 .+-. 2 55 .+-. 0 37 .+-. 6 IL-13 + COS 3 .+-. 3 5 .+-. 0 1 .+-. 1 <0.2 IL-13 + COS hCD40-L + antiIL-4 48 .+-. 8 167 .+-. 12 111 .+-. 7 48 .+-. 4 .+-. <0.2 8 .+-. 1 2 .+-. 0 <0.2 IL-13 <0.2 7 .+-. 0 1 .+-. 1 <0.2 IL-4 <0.2 4 .+-. 1 2 .+-. 0 <0.2	

Highly purified sIgD.sup.+ splenic B cells (5,000 cells/well) were cocultured with pJFE14 vector transfected or sorted COS7 cells (250 Cells/well) transfected with and expressing the human (h) or mouse (m) CD40L. IL13 (30 ng/ml) and IL4 (400 U/ml) were added as indicated. The sensitivities of the ELISAs (0.2 ng/ml for IgE and Igm, 0.4 ng/ml for IgG and total IgG) were determined with calibrated Ig standards (Behring, Marburg, Germany). \*No IgG determination was possible as there was detection of the Ig portion of CD40Ig fusion protein added.

**WEST**

Generate Collection

Print

L1: Entry 87 of 89

File: USPT

Oct 15, 1996

DOCUMENT-IDENTIFIER: US 5565321 A

TITLE: Detection of mutations in a CD40 ligand gene

**Brief Summary Text (13):**

The present invention also provides a method of utilizing gene therapy to correct X-linked hyper-IgM syndrome and other syndromes in which the CD40L gene does not encode biologically active CD40L. Gene therapy to correct such syndromes comprises isolating CD4+ T cells from an affected individual transfecting the isolated T cells with a transfection vector that expresses a biologically active CD40L, and administering the transfected T cells expressing biologically active CD40L to the individual.

**Detailed Description Text (7):**

To ensure that these mutations were not merely naturally occurring gene polymorphisms, two of the nucleotide changes found were introduced separately into a mammalian expression vector containing the complete coding region for the human CD40L using site-directed mutagenesis. Cells were transfected with vectors carrying either the wild type or the mutagenized CD40Ls, metabolically radiolabeled, and examined for expression of CD40L protein by precipitation with a polyclonal serum directed against CV1/EBNA cells expressing the human CD40L, or with CD40.Fc. Cells transfected with the wild type CD40L expressed a 33 kD protein that was detected using CD40.Fc. In contrast, cells transfected with either mutant form of CD40L did not express a protein recognized by CD40.Fc. Immunoprecipitation of identical lysates using the polyclonal antiserum resulted in the recognition of a 33 kD protein, from both mutant and wild type transfected cells, which co-migrated with the CD40L protein recognized by CD40.Fc. Northern blot analysis showed similar levels of CD40L-specific RNA in both wild type and mutant transfected cells. Additionally, cells transfected with either mutant form of CD40L were completely negative for CD40.Fc binding, while cells expressing the wild type CD40L showed strong CD40.Fc binding. Cells expressing either form of mutagenized CD40L were also unable to induce B cell proliferation or IgE secretion, confirming the absence of functional CD40L on their cell surfaces.

**Detailed Description Text (17):**

A gene encoding biologically active CD40L may also be introduced into T cells (preferably CD4+ T cells) obtained from an individual with abnormal or defective CD40L using gene transfer techniques. The cells are isolated, and transfected with the biologically active CD40L gene; they are subsequently re-administered to the individual, and will then correct the symptoms of the syndrome by producing biologically active CD40L.

**Detailed Description Text (38):**

To test whether the nucleotide changes detected in Example 3 affected the expression of the CD40L or its ability to bind to CD40, the two nucleotide changes found in Patient 1 and Patient 2 of Example 2 were introduced separately into a mammalian expression vector containing the complete coding region for the human CD40L using site-directed mutagenesis. A cloning vector containing human CD40L sequence, designated hCD40-L, was deposited with the American Type Culture Collection, Rockville, Md. (ATCC) on Dec. 6, 1991, under accession number 68873. Mutants were constructed using the gene splicing by overlap extension (SOEing) process (Horton et al., BioTechniques 8:528, 1990). The primer used to recreate the mutation found in Patient 1 was 5'-TGCGGGCAACATCCATTGCACTTGGGAGTATTGGAATTGCAA (SEQ ID NO:5). The primer used to recreate the mutation found in Patient 2 was

5'-CCATGAGCAACAACCTGGTAACCCCGGAAATGGGAAACAGC (SEQ ID NO:6). The remainder of the necessary primers were generated from the CD40L sequence (described in U.S. Ser. No. 07/969,703 and in Armitage et al., Nature 357:80, 1992 and Spriggs et al., J. Exp. Med. 176:1543, 1992). The resultant vectors were referred to as Mutant 1 and Mutant 2, respectively. The introduction of the appropriate nucleotide change was confirmed in the actual expression vectors by sequence analysis of the entire coding region. The human embryonic kidney cell line, 293, was transfected with vectors carrying either the wild type or the mutagenized CD40Ls, and on day 3 post transfection, cells were metabolically radiolabeled with <sup>35</sup>S Trans-label (ICN Radiochemicals, Irvine, Calif.). Cell lysates were prepared and examined for expression of CD40L protein by precipitation with a polyclonal serum directed against CV1/EBNA cells expressing the human CD40L, or with CD40.Fc. Cells transfected with the wild type CD40L expressed a 33 kD protein that can be readily precipitated using CD40.Fc. In contrast, cells transfected with either mutant form of CD40L did not express a protein recognized by CD40.Fc. Immunoprecipitation of identical lysates using the polyclonal antiserum, however, resulted in the recognition of a 33 kD protein from mutant as well as wild type transfected cells. This protein co-migrated with the CD40L protein recognized by the CD40.Fc and was not present in lysates transfected with vector alone. Consistent with these results, Northern blot analysis showed similar levels of CD40L-specific RNA in both wild type and mutant transfected cells.

#### Detailed Description Text (39):

Transfected cells were also examined by flow cytometric analysis. Cells transfected with either mutant form of CD40L were completely negative for CD40.Fc binding, while cells expressing the wild type CD40L showed strong CD40.Fc binding. To address the biological activity of the mutant CD40L proteins, cells transfected with wild type or mutagenized CD40Ls were examined for their ability to induce proliferation and IgE secretion from purified tonsil B cells co-cultured with IL-4. In contrast to cells transfected with wild type ligand (Table 1), cells expressing either form of mutagenized CD40L were unable to induce B cell proliferation or IgE secretion, confirming the absence of functional CD40L on their cell surface.

#### Detailed Description Text (43):

Previous work has shown that culture of single donor PBMC in the presence of IL-4 results in the production of IgE (IgE secretion from 1.times.10.sup.5 unfractionated to T-depleted PBMC was determined following 10 days culture with 5 ng/ml IL-4, together with 200 ng/ml G28-5 antibody (monoclonal antibody to CD40, obtained from Dr. E. A. Clark, University of Washington, Seattle, Wash.) or 1.times.10.sup.4 fixed CV1/EBNA cells transfected with vector alone or human CD40L. Preparation of PBMC and determination of secreted IgE concentrations were performed as described in Fanslow et al., J. Immunol. 149:655, 1992. Results are expressed as the mean +/- SEM of triplicate cultures. PBMC from normal donors (Controls 1, 3, and 4) produced measurable amounts of IgE when cultured with IL-4 (Table 2). In contrast, no IgE production was detected from any of the four hyper-IgM patients cultured under the same conditions. Significantly, in 3 out of 4 cases (Patients 1, 2, and 4), the addition of recombinant CD40L or the CD40 mAb, G28-5, to cultures containing hyper-IgM patients' PBMC restored their ability to secrete IgE. Similarly, the T-depleted PBMC (B-cell enriched cultures) from these three patients and from all controls examined secreted IgE in the presence of IL-4 plus either recombinant CD40L or G28-5 antibody (Table 2). In the case of Patient 3, no IgE was detected in PBMC cultured with IL-4 and recombinant CD40L or G28-5 antibody. The reason for these results is unclear. Additional PBMC were not available from this patient; thus, it was not possible to determine whether this lack of response was reproducible or due to experimental variation.

#### Detailed Description Paragraph Table (2):

TABLE 2

PBMC from hyper-IgM patients can secrete IgE IgE Secreted\* Patient/Control 1 2 3 4  
A.

Unfractionated PBMC Vector alone	Patient	<0.3	<0.3	<0.3	<0.3	Control	8.6	+. 1.2
		<0.3	16.3	+. 2.9	22.9	+. 3.7	CD40 ligand	Patient 43.3
		+. 4.8	51.0	+. 12.4				
		<0.3	33.5	+. 6.0	Control	73.4	+. 6.7	<0.3
		48.1	+. 4.4	25.7	+. 4.0	G28-5 mAb		
		Patient	76.4	+. 6.8	54.7	+. 12.5	<0.3	55.0
		+. 8.6	Control	87.1	+. 9.8	<0.3		
		87.2	+. 5.8	84.4	+. 8.2	B. T-depleted PBMC Vector alone	Patient	<0.3
		<0.3						



<0.3 Control <0.3 <0.3 <0.3 <0.3 CD40 ligand Patient 39.2 .+- . 4.4 22.6 .+- . 3.1  
<0.3 23.1 .+- . 6.3 Control 128.4 .+- . 13.6 <0.3 55.1 .+- . 4.6 80.2 .+- . 8.3 G28-5  
mAb Patient 69.4 .+- . 6.2 34.4 .+- . 11.9 <0.3 45.5 .+- . 6.0 Control 146.0 .+- . 12.2  
<0.3 93.5 .+- . 6.1 98.0 .+- . 9.5

secretion from 1 .times. 10.sup.5 purified tonsil B cells was measured after 10 days  
coculture with 5 ng/ml IL4, together with 200 ng/m G285 antibody or with CV1/EBNA  
cells transfected with CD40L or vector alone. \*IgE

**WEST**

Generate Collection



Print

L9: Entry 1 of 2

File: USPT

Mar 2, 1999

DOCUMENT-IDENTIFIER: US 5877210 A

TITLE: Phosphotyrosine phosphatase inhibitors or phosphotyrosine kinase activators for controlling cellular proliferation

**Brief Summary Text (37):**

(2) a method of treating a subject suffering from a malignant proliferative disorder selected from the group consisting of leukemias and lymphomas wherein proliferating malignant cells are selected from the group consisting of B cells, T cells, cells derived from malignant transformation of B cells or T cells, and myeloid cells, the method comprising the step of contacting the proliferating malignant cells with a coordinate-covalent complex as described above;

**Brief Summary Text (38):**

(3) a method of treating a subject suffering from a malignant proliferative disorder selected from the group consisting of leukemias and lymphomas wherein the proliferating malignant cells are selected from the group consisting of B cells, T cells, cells derived from malignant transformation of B cells or T cells, and myeloid cells, the method comprising:

**Drawing Description Text (14):**

FIG. 8 is a graph showing the inhibition by BMLOV of growth in mixed lymphocyte response cultures dependent on CD28 costimulation;

**Detailed Description Text (193):**

The data is shown in Table 2. The three B-cell lines, Ramos, Raji, and REH, were all highly sensitive to doses of 5 to 10 .mu.M BMLOV. The myeloid cell line THP-1 and the promyelocytic cell line HL-60 also were highly sensitive to BMLOV at a dose of only 1 .mu.M. BMLOV gave 99.8% clonogenic cell death for the Raji transformed B cell line at a dose of 10 .mu.M, and 99.4% clonogenic cell death for HL-60 promyelocytic leukemia cells at 1 .mu.M.

**Detailed Description Text (199):**

Ramos cells were highly sensitive to doses of 10 and 25 .mu.M BMLOV. The murine cell line BCL.sub.1 is a highly tumorigenic and lethal leukemia considered to be a model of human chronic lymphocytic leukemia (CLL). These cells were highly sensitive to the drug at a dose of 25 .mu.M. THP-1 cells showed only partial sensitivity to the drug at a dose of 25 .mu.M. In general, cells were more sensitive to the drug in the clonogenic assays than in the thymidine incorporation assay. These results suggest a greater requirement for phosphatase activity for cells to grow as colonies in methylcellulose relative to growth in free suspension in liquid media. The murine B cell lymphoma line A20, which forms highly aggressive tumors in mice, was very sensitive to a dose of 25 .mu.M of the drug. The human promyelocytic leukemia cell line HL60 and the human acute T cell leukemia cell line Jurkat were moderately sensitive to BMLOV at a dose of 25 .mu.M, whereas the human T cell acute lymphocytic leukemia CEM was resistant. These results indicate that malignant cells of B cell origin, including lymphoma, acute lymphocytic leukemia, and chronic lymphocytic leukemia are sensitive to BMLOV. Some leukemias of myeloid and T cell origin also show sensitivity to BMLOV whereas others are resistant.

**Detailed Description Text (217):**

Mixed lymphocyte response cultures that are dependent on CD28 costimulation were inhibited by over 50% by 0.5 to 5 .mu.M BMLOV (FIG. 8). These results suggest the phosphatase inhibitor may selectively block CD28 effects and therefore can be used

to block the generation of antibodies to antigens in animals.

Detailed Description Text (218):

An important question has been how does the costimulatory CD28 signal differ from the primary CD3 dependent signal in T cells, since both signals induce tyrosine phosphorylation and Ca<sup>2+</sup> flux. These results raise the possibility that the CD28 costimulatory or second signal requires a BMLOV-sensitive phosphatase activity that the primary signals do not.

Detailed Description Text (275):

The strong effects of BMLOV on B cell leukemia and lymphoma cells suggested that the effect of BMLOV on the growth of normal peripheral B cells be studied (FIG. 16A). Normal peripheral B cells require a mitogenic stimulus to grow, so the combination of anti-CD40 monoclonal antibody plus IL-4 was employed to induce proliferation, a combination that is known to result in prolonged proliferation (J. Banchereau et al., "Long-Term Human B Cell Lines Dependent on Interleukin-4 and Antibody to CD40," Science 251:70-72 (1991)). In FIG. 16A, normal peripheral B cells were cultured in RPMI media containing 10% fetal bovine serum and with the addition of 1 .mu.g/ml G28-5 plus 10 ng/ml IL-4 as indicated. Cells were grown for 5 days in the indicated concentrations of BMLOV and proliferation was measured by [<sup>3</sup>H]-thymidine incorporation. BMLOV treatment inhibited proliferation in a dose-dependent manner, but there was considerable variation in sensitivity between cells from two different donors (FIG. 16A). Nonetheless, at a dose of 10 .mu.M, the inhibition of proliferation of the normal B cells was markedly less than the strong inhibition observed for the transformed B cell lines (FIG. 15A).

Detailed Description Text (276):

In addition to promoting B cell proliferation, anti-CD40 antibodies are known to protect B cells in germinal centers from undergoing apoptosis (Y. Liu et al., "Mechanism of Antigen-Driven Selection in Germinal Centres," Nature 342:929-931 (1989)). The combination of IL-4 plus anti-CD40 has been reported to be more effective than either treatment alone in protecting B cells from apoptosis induced by hypercross-linking of surface IgM or IgD receptors (S. L. Parry et al., "Hypercross-linking Surface of IgM or IgD Receptors on Mature B Cells Induces Apoptosis that Is Reversed by Costimulation with IL-4 and Anti-CD40," J. Immunol. 152:2821-2829 (1994)). Accordingly, it was examined whether BMLOV induced cell deaths in the normal B cells (FIG. 16B). In FIG. 16B, normal peripheral B cells were grown for 4 days with the indicated concentrations of BMLOV and then stained with propidium iodine and analyzed for viability by flow cytometry. The percentage of cells in each quadrant is indicated. The lower right quadrant contains the viable cells. In the absence of any treatment, 67% of the normal B cells remained viable after 4 days in culture, whereas only 27% of the cells were viable following treatment with BMLOV (FIG. 16B). Treatment with G28-5, an anti-human CD40 monoclonal antibody, plus IL-4 protected the cells from the BMLOV induced death, with 58% of the cells remaining viable as opposed to 50% viability observed without BMLOV. In contrast to these results, treatment with combinations of anti-CD20 plus anti-CD40, phorbol ester plus IL-4, or phorbol ester plus anti-CD40 did not protect the cells from BMLOV induced death, even though these combinations are mitogenic for B cells (E. A. Clark & J. A. Ledbetter (1986), supra). Taken together, these results indicate that BMLOV can inhibit B cell growth in the absence of inducing cell death and suggest that BMLOV induced death can be blocked by appropriate biological stimulation that is known to protect against activation-induced cell death in vivo.

Detailed Description Text (277):

These results can be interpreted in view of the role of activation induced cell death in lymphocyte development. Activation induced cell death is extremely important to lymphocyte development, acting to remove self-reactive immature lymphocytes. Immature lymphocytes whose antigen receptors bind self antigen strongly and give a strong activation signal are eliminated by apoptosis (D. R. Green et al. (1992), supra). When lymphocytes mature, productive activation requires not only the primary stimulation of the cells' antigen receptor, but also costimulatory signals. In T cells, the binding of CD28 by its ligands of the B7 family serves this costimulatory role, whereas in B cells, costimulation occurs via binding of CD40 to its ligand gp39 (E. A. Clark & J. A. Ledbetter, "How B and T Cells Talk to Each Other," Nature 367:425-428 (1994)). In B cells, CD40 plays several important roles,

promoting proliferation, preventing apoptosis of germinal center B cells, and promoting immunoglobulin class-switching. In the absence of costimulation, lymphocytes that receive only antigen receptor stimulation become non-responsive or anergic and their growth is inhibited (E. A. Clark & J. A. Ledbetter (1994), supra).

**WEST**☐

Generate Collection

Print

L4: Entry 8 of 13

File: USPT

Nov 20, 2001

DOCUMENT-IDENTIFIER: US 6319709 B1

TITLE: Tumor cells with increased immunogenicity and uses therefor

Detailed Description Text (36):

The tumor cells to be modified as described herein include tumor cells which can be transfected or treated by one or more of the approaches encompassed by the present invention to express a costimulatory molecule. If necessary, the tumor cell can be further modified to express MHC molecules or an inhibitor of Ii expression. A tumor from which tumor cells are obtained can be one that has arisen spontaneously, e.g. in a human subject, or may be experimentally derived or induced, e.g. in an animal subject. The tumor cells can be obtained, for example, from a solid tumor of an organ, such as a tumor of the lung, liver, breast, colon, bone etc. Malignancies of solid organs include carcinomas, sarcomas, melanomas and neuroblastomas. The tumor cells can also be obtained from a blood-borne (ie. dispersed) malignancy such as a lymphoma, a myeloma or a leukemia.

**Set Name Query**

side by side

**Hit Count Set Name**

result set

*DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ*

<u>L10</u>	(CLL or chronic adj lymphocytic) same (cd40L or gp39 or cd40 adj ligand)	7	<u>L10</u>
<u>L9</u>	L8 and (c11 or chronic adj lymphocytic)	2	<u>L9</u>
<u>L8</u>	L7 and (costimulat\$) same (cd40L or gp39 or cd40 adj ligand)	12	<u>L8</u>
<u>L7</u>	(transfect\$ or transform\$) same (leukemi\$ or c11) and (costimulat\$)	264	<u>L7</u>
<u>L6</u>	L4 and (cd40L or cd40 adj ligand or gp39)	4	<u>L6</u>
<u>L5</u>	L4 and (c11 or chronic adj lymphocytic)	1	<u>L5</u>
<u>L4</u>	(transfect\$ or transform\$) same (costimulat\$) same (leukemi\$ or c11)	13	<u>L4</u>
<i>DB=PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ</i>			
<u>L3</u>	(transfect\$ or transform\$) same (costimulat\$) same (leukemi\$ or c11)	6	<u>L3</u>
<u>L2</u>	(cd40L or cd40 adj ligand or gp39 or 5c8) same (transfect\$ or transform\$)	76	<u>L2</u>
<i>DB=USPT; PLUR=YES; OP=ADJ</i>			
<u>L1</u>	(cd40L or cd40 adj ligand or gp39 or 5c8) same (transfect\$ or transform\$)	89	<u>L1</u>

END OF SEARCH HISTORY

**Set Name Query**

side by side

**Hit Count Set Name**

result set

*DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ*

<u>L10</u>	(CLL or chronic adj lymphocytic) same (cd40L or gp39 or cd40 adj ligand)	7	<u>L10</u>
<u>L9</u>	L8 and (c11 or chronic adj lymphocytic)	2	<u>L9</u>
<u>L8</u>	L7 and (costimulat\$) same (cd40L or gp39 or cd40 adj ligand)	12	<u>L8</u>
<u>L7</u>	(transfect\$ or transform\$) same (leukemi\$ or c11) and (costimulat\$)	264	<u>L7</u>
<u>L6</u>	L4 and (cd40L or cd40 adj ligand or gp39)	4	<u>L6</u>
<u>L5</u>	L4 and (c11 or chronic adj lymphocytic)	1	<u>L5</u>
<u>L4</u>	(transfect\$ or transform\$) same (costimulat\$) same (leukemi\$ or c11)	13	<u>L4</u>
<i>DB=PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ</i>			
<u>L3</u>	(transfect\$ or transform\$) same (costimulat\$) same (leukemi\$ or c11)	6	<u>L3</u>
<u>L2</u>	(cd40L or cd40 adj ligand or gp39 or 5c8) same (transfect\$ or transform\$)	76	<u>L2</u>
<i>DB=USPT; PLUR=YES; OP=ADJ</i>			
<u>L1</u>	(cd40L or cd40 adj ligand or gp39 or 5c8) same (transfect\$ or transform\$)	89	<u>L1</u>

END OF SEARCH HISTORY

**WEST**[Help](#)[Logout](#)[Interrupt](#)[Main Menu](#)[Search Form](#)[Posting Counts](#)[Show \\$ Numbers](#)[Edit \\$ Numbers](#)[Preferences](#)[Cases](#)**Search Results -**

Term	Documents
CLL.DWPI,EPAB,JPAB,USPT,PGPB.	1161
CLLS.DWPI,EPAB,JPAB,USPT,PGPB.	33
CHRONIC.DWPI,EPAB,JPAB,USPT,PGPB.	59970
CHRONICS.DWPI,EPAB,JPAB,USPT,PGPB.	3
LYMPHOCYTIC.DWPI,EPAB,JPAB,USPT,PGPB.	5731
LYMPHOCYTICS	0
CD40L.DWPI,EPAB,JPAB,USPT,PGPB.	548
CD40LS.DWPI,EPAB,JPAB,USPT,PGPB.	2
GP39.DWPI,EPAB,JPAB,USPT,PGPB.	207
GP39S	0
CD40.DWPI,EPAB,JPAB,USPT,PGPB.	1639
((CLL OR CHRONIC ADJ LYMPHOCYTIC) SAME (CD40L OR GP39 OR CD40 ADJ LIGAND)).USPT,PGPB,JPAB,EPAB,DWPI.	7

[There are more results than shown above. Click here to view the entire set.](#)

US Patents Full-Text Database  
US Pre-Grant Publication Full-Text Database  
JPO Abstracts Database  
EPO Abstracts Database  
Derwent World Patents Index

Database: IBM Technical Disclosure Bulletins

Search:

L10

[Refine Search](#)

[Recall Text](#)

[Clear](#)

**Search History**

DATE: Sunday, November 24, 2002 [Printable Copy](#) [Create Case](#)



**WEST**[Help](#)[Logout](#)[Interrupt](#)[Main Menu](#)[Search Form](#)[Posting Counts](#)[Show S Numbers](#)[Edit S Numbers](#)[Preferences](#)[Cases](#)**Search Results -**

Term	Documents
CLL.DWPI,EPAB,JPAB,USPT,PGPB.	1161
CLLS.DWPI,EPAB,JPAB,USPT,PGPB.	33
CHRONIC.DWPI,EPAB,JPAB,USPT,PGPB.	59970
CHRONICS.DWPI,EPAB,JPAB,USPT,PGPB.	3
LYMPHOCTIC.DWPI,EPAB,JPAB,USPT,PGPB.	5731
LYMPHOCTICS	0
CD40L.DWPI,EPAB,JPAB,USPT,PGPB.	548
CD40LS.DWPI,EPAB,JPAB,USPT,PGPB.	2
GP39.DWPI,EPAB,JPAB,USPT,PGPB.	207
GP39S	0
CD40.DWPI,EPAB,JPAB,USPT,PGPB.	1639
((CLL OR CHRONIC ADJ LYMPHOCTIC) SAME (CD40L OR GP39 OR CD40 ADJ LIGAND)).USPT,PGPB,JPAB,EPAB,DWPI.	7

[There are more results than shown above. Click here to view the entire set.](#)

Database: US Patents Full-Text Database  
US Pre-Grant Publication Full-Text Database  
JPO Abstracts Database  
EPO Abstracts Database  
Derwent World Patents Index  
IBM Technical Disclosure Bulletins

Search:

L10

[Refine Search](#)[Recall Text](#)[Clear](#)**Search History**

DATE: Sunday, November 24, 2002 [Printable Copy](#) [Create Case](#)